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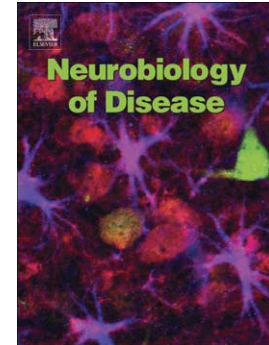
Neurotrophic factors and their inhibitors in chronic pain treatment

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Introduction

Chronic pain remains an area of considerable unmet medical need, as multiple statistics attest. For instance 6-8% of people in the UK are believed to suffer from neuropathic pain alone (Hall et al. 2013; Bouhassira et al. 2008). For all types of chronic pain, estimates of prevalence have risen to 20% (van Hecke, Torrance, and Smith 2013; Breivik et al. 2006). Critically, existing treatments for these pain states are limited with more than half of patients getting inadequate relief. The costs of all this persistent pain are enormous and relate not only to the suffering of the affected individuals, but often extend to their family and colleagues. The societal costs are conservatively estimated to amount to more than \$80 billion annually in the US (The national academies press 2011). This rather bleak situation is not without hope. In the last decades our understanding of the neurobiological mechanisms of chronic pain have advanced considerably. This new understanding is now beginning to be translated into therapy with a spate of positive phase II or III drug trials in chronic pain being reported in the last few years (Rice et al. 2014; Schwertner et al. 2013; Brown et al. 2012; Ford 2012). One of the most promising areas for developing novel analgesic drugs appears to be the targeting of neurotrophic factors, particularly nerve growth factor, and this is the topic of this review.

Mechanisms of persistent pain

To understand how neurotrophic factors might have an impact on pain perception, it is useful to first understand what processes underpin chronic pain states. Figure 1 illustrates schematically some of the major anatomical pathways involved. It shows that the peripheral tissues of the body are innervated by specialised sensory neurons. About two-thirds of these are dedicated nociceptors, which respond selectively to stimuli that damage or threaten to damage tissues. These nociceptive sensory neurons project into the CNS through the dorsal (posterior) roots and cranial nerves and terminate in the spinal and trigeminal dorsal grey matter which is the first site of integration of the nociceptive information. Output neurons carry nociceptive information to several brainstem sites and from there to a distributed series of cortical areas. The spinal and trigeminal relays are also subject to multiple inhibitory and excitatory descending controls. The interested reader is referred to more extensive reviews of these much studied pathways (see (Schaible 2006; Dubin and Patapoutian 2010; A. Patapoutian, Tate, and Woolf 2009)). However, the initial stages of processing are particularly important in relation to the role of neurotrophic factors and pain. The key point to understand is that the activation of peripheral nociceptors and spinal transmission of nociceptive information is greatly amplified by a variety of forms of tissue injury and many disease processes. That is, chronic pain is not just the repetition of acute pain but is associated with a sensitization of the pain-signalling pathway. Two forms of sensitization have been intensively studied over the decade or so, and are believed to be a major contributor to clinically relevant pain states.

The first form is generally known as peripheral sensitization, and as the name suggests, this represents a dynamic change in the responsiveness of nociceptors to applied stimuli, so that for instance, skin temperatures of 40°C rather than 45°C activate some fibres. We have a fairly good understanding of the molecular basis for this sensitization, as reviewed in chapter 3 (McMahon et al. 2013). Many molecules can induce this sensitization including some neurotrophic factors, as we describe below.

The second form of sensitization occurs within the spinal cord and is known as central sensitization. In this case, post-synaptic cells that relay information supraspinally produce an augmented output for a given level of peripheral stimulation, and in some cases begin to respond *de novo*. Again, the molecular pharmacology of this process has been intensively studied. We review below how transcriptional regulation of primary nociceptive neurons can contribute to this process of central sensitization and this demonstrates a second way in which some neurotrophic factors acting on the peripheral terminals of nociceptors can contribute to chronic pain. An intriguing feature of this

sensitisation is that for some pain states, particularly neuropathic one, a role of spinal microglia appears obligatory. That is, these resident macrophages of the CNS shift from a resting state to one in which they release a number of inflammatory mediators (including some neurotrophic factors) and these in turn are critical for the development of the central sensitised state (Schaible 2006; Latremoliere and Woolf 2009).

Recently a third mechanism has been recognised that may be an important – and neurotrophic factor dependent – contributor to persistent pain states. This is the proliferation of nociceptive axons and terminals in peripheral target tissues. It has been known for a long time that cartilage is normally aneural but that in patients with osteoarthritis thin calibre nerve fibres begin to be seen in the tissue. In a series of elegant studies, Patrick Mantyh and his colleagues have studied an animal model of bone cancer pain. They have shown that one feature of the model is a dramatic increase in small fibre innervation of the tumour in bone. They also show this is dependent on tumour-derived overexpression of one trophic factor, NGF, and that blocking this factor eliminates the hyper-innervation and the sensory abnormalities seen in this model (see below) (McCaffrey et al. 2014). There are other chronic pain conditions associated with increased neural innervation such as endometriosis (McKinnon et al. 2015). Neurotrophic factors are of course by definition molecules that have the potential to regulate the growth of neurons, unlike the majority of other known pain mediators. The relative importance of this mechanism vs. peripheral and central sensitization is not yet clear.

We now turn to the evidence for and pro-nociceptive mechanisms of, specific neurotrophic factors.

Nerve growth factor (NGF) as a pain mediator

Nerve growth factor (NGF) is a member of the neurotrophin (NT) family of growth factors (GF) which also include BDNF (brain derived neurotrophin factor), neurotrophin-3 and 4/5 (NT-3 and NT-4/5). It was first discovered in 1948 by Elmer Bueker, confirmed by Rita Levi-Montalcini (Cohen, Levi-Montalcini, and Hamburger 1954) (Levi-montalcini 1987) and then later isolated by Stanley Cohen in 1960 from the submandibular gland in mice (Cohen 1960). NGF was the first member of the NT family to be positively identified, with BDNF and NT-3 isolated subsequently. Over the past 60 years our understanding of this molecule and its role in disease has advanced significantly.

NGF, TrkA and p75^{NTR}

NGF signals via two receptors, the high affinity TrkA receptor and a lower affinity p75 neurotrophin receptor (p75^{NTR}) (summarised in Figure 2).

TrkA is a member of the tropomyosin family of tyrosine receptor kinases. It is a transmembrane spanning protein with a highly conserved tyrosine kinase domain in the intracellular region, helical transmembrane domain and a leucine/cysteine rich extracellular domain containing an IgG-C2 domain. Trk receptors have a high specificity for a particular member of the NT family where TrkA is selective for NGF, (with some activation by NT-3). TrkB is selective for BDNF and NT-4/5 and TrkC selective for NT-3. In the presence of NGF the TrkA receptor monomers dimerise and the intracellular part of the receptor becomes autophosphorylated which leads to the activation of a number of growth and survival signalling pathways via MAPK, AKT and PLC γ (Pezet and McMahon 2006; Kiris et al. 2014; Wiesmann et al. 1999).

p75^{NTR} is a member of the tumour necrosis factor receptor 1 (TNFR1) superfamily and is described as a promiscuous receptor as it is capable of interacting with all members of the NT family. Like the Trk receptors, p75^{NTR} is also a transmembrane protein consisting of a helical transmembrane domain. However, the extracellular domain contains four cysteine rich repeat regions and a highly conserved

unique intracellular domain which includes a death domain region. Therefore, signalling of NGF via the p75^{NTR} has been described to activate apoptotic pathways and exert its effect via JNK and NF- κ B, however when co-expressed with TrkA it can promote growth (Meeker and Williams 2014; He and Garcia 2004).

However, the distinction of NGF signalling via TrkA for growth and survival and via p75^{NTR} for activation of apoptotic pathways is a simplified view of the signalling pathways involved. Further investigation and greater understanding of NGF signalling has thus far uncovered a more complex signalling process.

One complication is that NGF is transcribed as a pro-form (proNGF) which is cleaved to release a mature NGF. It now appears that both the proNGF and mature NGF can be released by cells, and the pro-form appears to be a selective agonist for p75^{NTR} (Hempstead 2009). In addition, a number of other receptors have been identified which modulate NGF signalling. Sortilin for example, a receptor mainly associated with endosomes can control the trafficking of receptors such as TrkA to the cell surface, but in addition, has also been shown to interact with the p75^{NTR} as a co-receptor for proNGF signalling (Capsoni and Cattaneo 2006; Meeker and Williams 2014). Other studies suggest that the Nogo receptor can interact with the p75^{NTR} and modulate its signalling, or that activation of G-protein coupled receptors can transactivate TrkA in the absence of NGF (Meeker and Williams 2014). Finally, despite the two receptors controlling opposing signalling pathways there is evidence that the TrkA and p75^{NTR} can interact and modulate the downstream signalling (Meeker and Williams 2014). Hence, NGF signalling can be complex, and depend on both the form of NGF present and the pattern of different receptors present on a cell. What is clear however is that many of the biological actions of NGF do depend critically on signalling through TrkA (Patapoutian and Reichardt 2001).

In the adult nervous system, TrkA is expressed on peptidergic C fibres, sympathetic post-ganglionic neurons and small populations of CNS neurons (Usoskin et al. 2014; Thakur et al. 2014). There are also claims that multiple immune cells express TrkA including mast cells, macrophages, satellite glial cells (SGC), T and B cells (Aloe et al. 2012). However, these latter claims are mostly not supported by recent genome wide sequencing studies (Gosselin et al. 2014; Lavin et al. 2014; Mo et al. 2015).

p75^{NTR} has a more widespread expression pattern, co-expressed with TrkA on peptidergic nociceptors and other sensory neurons, and in moderate amounts on other cell types including sympathetic post-ganglionic neurons, neutrophil granulocytes, macrophages (Richner et al. 2014; Aloe et al. 2012) and Schwann cells surrounding sensory axons bundles of C fibres (Ebenezer et al. 2007; Aloe et al. 2012). Surprisingly, there is little expression in the CNS and, as with TrkA, expression on immune cells is not well supported in recent genome wide sequencing studies (Gosselin et al. 2014; Lavin et al. 2014; Mo et al. 2015).

Role of NGF in development

NGF is essential for the development and survival of sensory and sympathetic neurons (Patel et al. 2003). Knockout of NGF in mice showed that despite survival *in utero*, knockouts do not survive past weaning with a maximum lifespan of 4 weeks. Compared to littermates, NGF null mice display developmental delays in hair growth and ptosis and a marked insensitivity to noxious stimuli in addition to a severe loss of cells in the superior cervical ganglia (SCG) and a 70% loss of neurons in the dorsal root ganglia (DRG), specifically small diameter neurons (Crowley et al. 1994). Transgenic mice with a knockout for the TrkA receptor show a similar phenotype to the NGF knockout mice. As with the NGF knockout mice, TrkA knockouts are viable *in utero* surviving to a maximum of 55 days post-natal. Sensory defects measured by a failure to detect deep pinpricks in whiskers and rear paws and thermal hyposensitivity are apparent by post-natal day 10 (P10) and at 3-4 weeks of age there are indications of self-mutilation (Smeyne et al. 1994). These mice also show a loss of neurons in the

SCG and a 70-90% loss of DRG neurons, preferentially small diameter neurons (Smeyne et al. 1994). In contrast, mice lacking the $p75^{\text{NTR}}$ are born physically normal and are fertile. Mice are observed to have a loss of epidermal nerve fibres including those positive for calcitonin gene-related peptide (CGRP) (Lee et al. 1992; Bergmann et al. 1997) and have a deficit in thermal sensitivity (Lee et al. 1992) and mechanical sensitivity (Bergmann et al. 1997) with some evidence of self-mutilation in hind paws by 4 months of age (Lee et al. 1992). Although there is no indication of any effect in SCG (Lee et al. 1992), DRGs appear noticeably smaller however there is no significant loss in a particular population of neurons (Bergmann et al. 1997). Overall, this suggests that NGF signalling via the TrkA receptor plays a dominant role in development.

Evidence from human studies further suggests a role for NGF in development. Hereditary sensory and autonomic neuropathy (HSAN) IV and V patients exhibit insensitivity to pain as a result of an improper nervous system development. HSAN IV (also known as CIPA – chronic insensitivity to pain with anhydrosis) arises from a mutation in the *NTRK1* gene encoding the TrkA receptor. It was first described in 1996 when testing of multiple families of CIPA patients showed mutations resulting in a frameshift or an alternate splice site in exons 1-3 (Yasuhiro Indo et al. 1996). Since then multiple mutations have been discovered across the 17 exons of TrkA (for review see (Y Indo 2012)). NGF is a key regulator during the development of the sensory and sympathetic nervous system and in maintenance of the cholinergic neurons in the basal forebrain (Capsoni and Cattaneo 2006). As a result patients with HSAN IV show symptoms of insensitivity to pain, anhydrosis and mental retardation (Yasuhiro Indo et al. 1996) with some indication of an immune phenotype (Beigelman et al. 2009). HSAN V patients exhibit insensitivity to pain without anhydrosis or mental retardation as a result of a mutation in the *NGFB* gene encoding NGF (Capsoni et al. 2011). However, a mutation in the *NGFB* gene has been reported in a Bedouin family causes a frameshift in the *NGFB* gene, which results in signs of a HSAN IV phenotype despite not carrying a mutation in the *NTRK1* gene (Carvalho et al. 2011).

Changing role of NGF post-natally, role in the adult

Although there is a clear role for NGF in development, there is a change in its role post-natally. It has been described that from P3 to P14 there is a downregulation in TrkA expression from 80% of DRG neurons to 40-50% of neurons, which correlates with an increase in the expression of Isolectin B4 (IB4) positive neurons – a marker of non-peptidergic C fibres (Bennett et al. 1996). This change in NGF dependence and differentiation to peptidergic/non-peptidergic C fibres from early developmental events hinges on *Runx1* expression (Lallemend and Ernfors 2012). Fully differentiated neurons which are NGF independent (for survival) are established (in the rodent) by P14. Most importantly these mature sensory neuron are not dependent on NGF for survival.

Functional role of NGF on the adult nociceptive system

In the adult the most well described role for NGF is in the development of persistent pain. This is supported by a number of animal studies using pharmacological methods of targeting the NGF/TrkA pathway. It has been reported that treatment of mice or rats with anti-NGF or a TrkA blocker results in an attenuation of thermal and mechanical allodynia in a wide range of animal models of persistent pain (Bennett et al. 1998; McMahon et al. 1995; Woolf et al. 1994; Lewin, Ritter, and Mendell 1993). In regards to human disease NGF has been linked to a number of different persistent pain conditions including osteoarthritis (OA), lower back pain (LBP), diabetic peripheral neuropathy (DPN), bladder pain syndrome (BPS), bone cancer pain and endometriosis (Pecchi et al. 2014; Bannwarth and Kostine 2014; Aloe et al. 2012; Capsoni and Cattaneo 2006; McCaffrey et al. 2014; Alvarez and Levine 2014; Jiang, Liu, and Kuo 2014).

NGF plays an important role in the development and maintenance of chronic pain in adult animals. Firstly, NGF has a role in the development of peripheral sensitivity. Following peripheral injury or

inflammation, there is recruitment of immune cells to the injury site and the body responds by secreting multiple factors including growth factors (e.g. NGF), cytokines and chemokines as part of an “inflammatory soup”. Binding of NGF, and other sensitising compound present in the “inflammatory soup”, (Bradykinin, PGE₂, TNF α and endothelin to name a few), to receptors on peripheral nociceptors induces rapid sensitisation of the nociceptive response (A. Patapoutian, Tate, and Woolf 2009; Dubin and Patapoutian 2010). NGF (unlike Bradykinin or other sensitising molecules) is also retrograde transported back to the cells soma where it can induce rapid transcript of many pain related genes. Some of these, (TRPV1 for example), are transported back to the periphery to reinforce peripheral sensitisation (A. Patapoutian, Tate, and Woolf 2009). Others, such as BDNF, are packed into dense core vesicles and released in response to activity from central processes on nociceptors, contributing to central sensitisation.

Intrathecal injection of NGF has been shown to increase the expression of BDNF in TrkA positive DRG neurons to almost 85% neurons expressing BDNF, compared to a minimal increase in BDNF expression in TrkB or C positive DRG neurons (Kerr et al. 1999; Michael et al. 1997). In addition, intrathecal NGF results in an increase in the number of axons expressing BDNF in the spinal cord (Michael et al. 1997; Kerr et al. 1999), projecting into laminae I and II from dorsal roots, which also appear to co-express CGRP, another typical marker of TrkA positive neurons (Michael et al. 1997). Further to this, BDNF has been shown to enhance the effect of NMDA receptor on central sensitisation in a model where rats were pre-treated with NGF prior to injection with a TrkB-IgG antibody a reduction in the formalin second phase was observed, which is also linked to the process of central sensitisation (Kerr et al. 1999).

One other mechanism whereby NGF might cause pain by promoting sprouting of nociceptive fibres in peripheral tissues. NGF has a well-established role in promoting growth of sensory and sympathetic neurons *in vitro* and *in vivo*. In several pain states, for example bone cancer pain, there is an apparent effect for NGF to promote the sprouting of axon terminals of nociceptive neurons resulting in a hyper-innervation of peripheral tissue. It is unclear what functional consequence this has on the nociceptors, but it could result in an increased responsiveness nociceptors to stimulation and thus an increase in pain. In bone cancer pain for example sprouting of CGPR positive fibres can be blocked by early treatment with anti-NGF (Jimenez-Andrade et al. 2011). In addition, anti-NGF treatment in a mouse model of bone cancer pain showed mice treated resulted in attenuated guarding and flinching behaviour in anti-NGF treated mice compared tumour only controls (Sevcik et al. 2005; Jimenez-Andrade et al. 2011; McCaffrey et al. 2014). This was accompanied by a reduction in markers of peripheral sensitisation – ATF-3 and CD-68 positive macrophages in the DRG – and central sensitisation – dynorphin-IR and c-fos in deep laminae of the spinal cord (Sevcik et al. 2005).

Overall, treatment with an anti-NGF showed an attenuation of pain related behaviours in accordance with a reduction in peripheral and central sensitisation markers and reversal of hyper-innervation of peripheral tissues suggesting that NGF plays a diverse role in the adult nociceptive system. These effects are summarised in Figure 3.

Therapeutic potential of anti-NGF in experimental and clinical studies

Given the range of biological effects of NGF on the nociceptive system, it is not surprising that multiple studies have explored the importance of this factor as a pain mediator in a variety of experimental pain models. These studies began in the 1990's and are still being performed today. The early studies focussed on inflammatory models induced for instance by carrageenan or CFA (e.g. (Woolf et al. 1994; McMahon et al. 1995)). Over the years the range of models tested has increased dramatically and one of the striking features of this literature is the consistency of anti-NGF effects. There is good preclinical evidence for a role of NGF in acute burn injury, UV inflammation, arthritis and bone cancer as an effect analgesic to name a few (for review see (Pezet and McMahon 2006)).

Following these findings in preclinical models a number of anti-NGF therapies developed for the clinic, which have been progressed into phase III clinical trials, mainly in OA patients. Multiple anti-NGF monoclonal antibodies are currently in phase III trials including tanezumab (Pfizer), fasinumab (Regeneron) and fulranumab (Janssen and Regeneron). Typically, anti-NGF in clinical trials is administered as part of a combined therapeutic approach with a non-steroidal anti-inflammatory drug (NSAID), the current treatment course for OA patients. However, due to an increase in the number side effects reported, including cases of rapidly accelerated OA (RPOA) and osteonecrosis in both affected and unaffected joints of OA patients clinical trials were put on hold in 2010 by the FDA, and re-evaluated in 2012 (Hertz and Fields 2012; Holmes 2012). As of March 2015 the hold on clinical trials was lifted on most of the OA trials with the condition of better screening for adverse effects including to the autonomic nervous system (Mullard 2015). The incidence of rapidly progressing OA seems to be associated with combined use of anti-NGF and NSAID treatment, so the new trials will focus on anti-NGF monotherapies

Despite side effects, results from clinical trials showed a robust reduction in pain compared to placebo in patients in a variety of conditions. Tanezumab has been most widely tested, showing a striking dose dependent effect on reducing pain in OA, with a reduction in pain scores from 20-50% with increasing doses (Lane et al. 2010) (see Figure 4). This striking effect has been repeated in a number of other subsequent trials. In addition to OA, there have also been beneficial effects of anti-NGF observed in LBP, DPN and bladder pain syndrome *in vivo* and clinical trials (Bannwarth and Kostine 2014; Capsoni and Cattaneo 2006; Aloe et al. 2012). This includes fulranumab treatment in patients with DPN which showed a dose dependent alleviation of pain despite early termination of the phase II clinical trial in response to the FDA hold (H. Wang et al. 2014). A meta-analysis of anti-NGF treatment in LBP patients also indicates that anti-NGF has a beneficial role in alleviation of pain and functional recovery (mainly tanezumab), although after screening over a thousand studies, only 4 randomised controlled trials were analysed, which further implicates a need for further research into the effects of anti-NGF (Leite et al. 2014).

There have also been some negative clinical trials with anti-NGF agents. These include chronic pancreatitis, endometriosis and painful intervertebral discs (for review see (Pezet and McMahon 2006)). Of course, we do not know if these negative findings result for patient heterogeneity, trial design issues or because NGF is not an important pain mediator in these conditions. The range of effect sizes seen in different anti-NGF trials suggests that other important pain mediators beyond NGF contribute to clinical pain states and this is itself intuitively plausible. Further clinical trials will be necessary to clarify the importance of NGF in different pain states.

Overall, both *in vivo* and clinical trials show anti-NGF to be a highly effective as an analgesic therapeutic (Bannwarth and Kostine 2014). It is not yet a licenced product, and the adverse effects reported may frustrate full development of anti-NGF. However, since there are multiple opportunities to interfere with NGF signalling it is likely that some of these will be explored for potential analgesic effects, perhaps with less of a side effect profile (since the mechanism of RPOA is not known).

Current questions around NGF

Despite an extensive amount of research into functional and pharmacological effects of NGF since it was isolated there are still a number of unanswered questions that need to be addressed.

Although it has been established that there is a role for NGF in pain through a number of mechanisms, it is still unclear what the relative importance of these are. As described above, various cells in the PNS and CNS are said to express the TrkA and/or p75^{NTR} and secrete NGF and/or other sensitising components of the “inflammatory soup”. Therefore, it is reasonable to question

whether NGF sensitisation of sensory neurons is a direct effect of NGF on the neurons themselves, or is there an indirect effect via another cell type. In addition there is some debate as to how NGF signals through its two different receptors. From a simplified view, NGF signalling on TrkA promotes survival and growth where signalling through the p75^{NTR} promotes apoptosis. However, almost 60 years on from the discovery of NGF has only added complexity to the signalling process, with more potential contributions and subtle alterations to the signalling pathway coming from a number of different sources.. It is possible that either the TrkA or p75^{NTR} could have the greatest effect or there could be subtle modulation of signalling between the two receptors. Finally, it is unclear what is the contribution of proNGF versus the mature NGF form in the NGF signalling pathway.

It has been well established that OA patients treated with anti-NGF has a beneficial effect on pain above that of the current pain management options. However, despite multiple clinical trials it is still undergoing testing due to a number of unexplained side effects. Questions such as what is causing these underlying adverse side effects and the suggested impact on the autonomic nervous system require further investigation. What is there about the anti-NGF treatments that make it such an effective treatment? There are a number of other components of the “inflammatory soup” that could be a useful target for chronic pain therapies but what makes NGF so effective. Could it be the result of a multi-action effect on peripheral and central sensitisation and, in the case of bone cancer pain, in some cases reversal of hyper-innervation of tissue?

In conclusion, over 60 years of research has shown that NGF is essential in development in sensory and sympathetic neurons, but in the adult there is a role for NGF in nociceptive pain, through several different mechanisms. *In vivo* models and more recently clinical trials have shown that treatment with anti-NGF is highly effective in the alleviation of pain, specifically in OA, over and above currently prescribed treatments. If the side effect profile of anti-NGF can be overcome or mitigated, it is likely that this treatment could form an important addition to analgesic armoury for patient use

IL6 as a pain mediator

While the evidence for NGF is extremely compelling – in both experimental models and some clinical pain states, it is worth noting that blocking NGF has been found to have quite limited or no efficacy in other pain states. The possibility that IL6 may also act as a pain mediator has been growing, although its role remains largely untested in the clinic.

Interleukin-6, identified more than three decades ago, was known predominantly as a T-cell derived cytokine responsible for activating and converting B cells into antigen-producing B cells. Several years on, and , IL6 is now recognised as a pleiotropic haematopoietic-neuropoietic cytokine, which exhibits both inflammatory and anti-inflammatory effects, along with playing a role in the survival of blood cells and neurons (Wierzbowska, Urbańska-Ryś, and Robak 1999). The central role played by IL6 in various host defence mechanisms and its position in the frontline of protection against injury and infection cannot be disputed (Akira et al. 1994; Scheller et al. 2011). There is considerable current interest in understanding the physiological effects of IL6 is predominantly due to its involvement in the pathology of several disorders such as Multiple Myeloma, Rheumatoid Arthritis, Osteoporosis, and Kaposi’s sarcoma amongst many others (Simpson et al. 1997). IL6 has also been strongly implicated in several neuropathic disorders, where its expression is found to be drastically increased (Murphy et al. 1999).

There is considerable circumstantial evidence for the involvement of IL6 in pain states in humans, but definitive data is still lacking. This includes evidence that IL6 can promote pain in humans. This is supported by studies showing substantial increase in levels of IL6 following injury or disease. Unlike NGF, there is clear evidence for increased IL6 expression within the CNS in several conditions. While IL6 is classified as a cytokine, it also clearly acts as a neurotrophic factor, promoting cell growth and

preventing cell death in some circumstances and the evidence supporting its role as a pain mediator is therefore reviewed here.

IL6 and its Receptor complex

The effect of IL6 is mediated through its interaction with, firstly, a type 1 transmembrane protein, IL6R (gp80). However, for the process of signalling to be initiated, the IL6-IL6R complex needs to bind to another transmembrane protein known as gp130. IL6R forms the ligand-binding α -subunit, while gp130 forms the signal transducing β -subunit of the complex (Gearing et al. 1992; Taga and Kishimoto 1997). The discovery of gp130 as a potential receptor for IL6 signalling was based on the finding that IL6R has a very small cytoplasmic tail, only around 82 amino acids, and IL6 could continue to signal through the IL6R despite the deletion of the cytoplasmic region (Yamasaki et al. 1988). This led to the identification of a 130k-Da membrane glycoprotein, gp130, which was found to be upregulated in cells after stimulation with IL6. However, gp130 by itself is incapable for signalling for IL6 but its presence is essential for the formation of a high-affinity receptor that contains the IL6 and IL6R complex (Taga and Kishimoto 1997). In contrast to the IL6R, gp130 is ubiquitously expressed in all cell types (Hibi et al. 1990).

Interestingly, most of the cells in the human body do not express the IL6R and hence are unable to respond directly to IL6 stimulation. However, a soluble form of the receptor, the sIL6R that is generated either by the proteolytic shedding of the membrane bound IL6 receptor or by a differently spliced mRNA species is responsible for the signalling of IL6 in many of these cells (Müllberg et al. 1993; Horiuchi et al. 1994). Yasukawa et al (1990) showed that the sensitivity of cells expressing both IL6R and gp130 to IL6 was further increased in the presence of the soluble IL6 receptor (sIL6R) (Yasukawa et al. 1990). Additionally, a naturally occurring secreted version of the IL6R has been reported in human serum and urine (Novick et al. 1989; Frieling et al. 1994). Healthy individuals contain around 80ng/ml of sIL6R in the serum, and this is increased under certain disease states such as HIV and multiple myeloma (Narazaki et al. 1993; Honda et al. 1992). This is in accordance with the levels of IL6 seen, which is almost undetectable in healthy individuals but increases substantially in patients with Rheumatoid Arthritis, Multiple myeloma, bacterial infections and HIV (Akira and Kishimoto 1992). Scheller et al (2011) tried to resolve the paradox surrounding the pro- and anti-inflammatory roles of IL6 by suggesting that classical signalling via the membrane bound IL6R is responsible for the regenerative properties of IL6, where trans-signalling via the sIL6R are behind the pro-inflammatory effects of IL6.

IL6 and chronic pain

Several convincing studies over the past few years have shown that neurons are not the only players involved in various clinical pain states. The interactions between the immune system and the neurons play an important role in how the peripheral and central nervous systems respond to peripheral nerve injury. The neuro-inflammatory response, particularly the involvement of inflammatory cytokines such as TNF- α and IL6, that are both released following nerve injury, have been associated with the sensitization of the central nervous system and the development of chronic pain (Scholz and Woolf 2007; Moalem and Tracey 2006). A comparative cross-sectional study of pro-inflammatory markers in chronic neuropathic pain patients showed a significantly increased presence of IL6 in these individuals as compared to controls, while other inflammatory markers such as IL-1 β , IL-8 and GM-CSF were not affected (Bäckryd et al. 2016). Under normal conditions IL6 can be produced by developing peripheral sensory and sympathetic neurons. However in adulthood IL6 production is almost completely inhibited in these cells, except under pathological condition (Zhong et al. 1999; Gadiant and Otten 1997). There is ample evidence implicating IL6 in the pathogenesis of

several neurological diseases and this is attested by the widespread presence of IL6 and IL6R in the nervous system, suggesting its potentially important physiological and functional role.

IL6 is expressed in the CNS in a number of pathological conditions including Parkinson's disease, Alzheimer's disease, spinal cord injury and Lupus patients with CNS involvement. Peripherally, post-natal expression of IL6 and IL6R mRNA has been found in superior cervical ganglia as well as the dorsal root ganglia (Gadient and Otten 1997). Interesting, even though most neurons express IL6, they are usually unable to respond to any IL6. März et al (1998) showed that this could be due to the restricted amount of IL6R that is present on the neurons, and the ability of the neurons to respond to IL6 could be increased by the providing exogenous soluble IL6R (März et al. 1998). The role of IL6 in promoting, controlling and modulating various inflammatory pain states is fairly well researched and established (De Jongh et al. 2003). Apart from the presence of increased IL6 levels in painful conditions that has been reported by several studies, the level of IL6 is proportional to the pain intensity over time (Yan et al. 2012). IL6's direct action on sensory neuron nociceptors was made evident by studies conducted by Andratsch et al (2009) who were successful in showing that knock out of the IL6 co-receptor from sensory neurons significantly reduces their nociceptive sensitization (Andratsch et al. 2009).

Pharmacological and Functional effects of IL6

Several studies have been successful in showing that IL6 can support and enhance the survival of cultured primary sympathetic neurons, septal cholinergic neurons and mesencephalic catecholaminergic neurons (Hama et al. 1991), retinal ganglion cells (Torres and de Araujo 2001) and dorsal root ganglion neurons (Thier et al. 1999). This effect of IL6 was intensified in the presence of the sIL6R (Reviewed in (Erta, Quintana, and Hidalgo 2012)).

The pro-nociceptive properties of IL6 have been assessed in several experimental studies where intraplantar, intracerebroventricular, and intrathecal injections of IL6 have been shown to cause excessive allodynia and hyperalgesia in various laboratory animals (Oka et al. 1995; Poole S, Cunha FQ, Selkirk S, Lorenzetti BB 1995; DeLeo et al. 1996). Furthermore, IL6 along with its soluble receptor (sIL6R) are capable of modulating production of other neurotrophic factors as well (Oh et al. 1998).

Effects of IL6 on central sensitization

Looking specifically at the impact of IL6 application centrally, Yan et al (2012) suggested the involvement of IL6 in migraine pain, by showing that IL6 can sensitize the dural afferents by modulating neural excitability via the sodium channel Nav1.7. They showed that meningeal application of IL6 in rats can lead to what they considered migraine like-pain. Additionally, apart from increased peripheral sensitivity following IL6/sIL6R administration, injection of IL6 along with its co-receptor is capable of causing central sensitization as well. Vazquez et al (2012) showed that injecting IL6/sIL6R into the knee joints of rats lead to increased spinal excitability along with that at the site of injection, as quickly as 1 hour post injection (Vazquez et al. 2012). Another recent study by Mukaino et al (2010) used anti-IL6-R antibody to block IL6 signalling and showed that neutralizing the IL6 pathway can lead to quicker motor recovery of rats with Spinal cord injury (Mukaino et al. 2010). The importance of this can be judged from several other reports in the past that have consistently indicated that Spinal cord injury is linked with increase in IL6 production, which is also positively correlated with development, as well as severity, of pain in both animal models of SCI as well as in patients (Davies, Hayes, and Dekaban 2007; Detloff et al. 2008). Furthermore, a humanized monoclonal anti IL6 R antibody, tocilizumab, that is approved for use in RA patients, was used in a recent study by Ohtori et al (2012) to treat patients with sciatica (Ohtori et al. 2012). They were successful in showing that application of tocilizumab on the spinal nerve of these patients alleviated pain to a higher degree than treatment with dexamethasone.

Moving into the periphery, it is interesting to note that while IL6 expression levels are almost negligible in healthy human beings, its expression can be significantly upregulated under painful inflammatory diseases (SMITH et al. 2001; Kiefer et al. 2001). This led to a plethora of studies attempting to investigate the effect of exogenous IL6 administration in mouse models. Direct injections of IL6 into rats causes a dramatic increase in thermal and mechanical sensitivity at the site of injection (Brenn, Richter, and Schaible 2007; DeLeo et al. 1996; Flatters, Fox, and Dickenson 2003). As discussed above, IL6 usually requires co-administration of the sIL6R in order to elicit the required effects. Andratsch et al (2009) demonstrated that direct injections of the IL6/sIL6R fusion protein complex, Hyper IL6 (HIL6), into the footpad of mice can lead to a significant decrease in the paw withdrawal latencies to heat stimulation. They also went on to further validate the effect of IL6 by showing that other gp130 cytokine activators such as LIF and OSM are incapable of producing the thermal hypersensitivity that is produced following IL6 treatment. Previously, Zhang et al (1999) showed that IL6^{-/-} mice elicit significantly lower compound action potentials from the sensory branch as compared to normal wildtype mice. They also went on to show that the IL6^{-/-} mice had a reduced sensitivity to temperature. Furthermore, IL6 is required for the regeneration of peripheral nerves under normal conditions as IL6^{-/-} animals recover more slowly, and never completely from crush-lesioned sciatic nerve injury (Zhang and Adachi 1999). A recent study trying to elucidate the effect of IL6 in Antigen-induced arthritis used mice lacking the main signalling receptor of IL6, gp130, in primary sensory neurons. They demonstrated that the mice lacking the signalling receptor for IL6 showed a dramatic reduction in the knee-joint swelling and also in the release of neuropeptides such as CGRP that are associated with pain and neuronal sensitization, in comparison to mice that ubiquitously expressed gp130 in their primary sensory neurons (Ebbinghaus et al. 2015) (see Figure 5).

To conclude, research over the past few years has made it evident that the central nervous system is not 'immune' to regulation by the immune system, as was previously believed. It is now well established that the nervous and the immune system can play a significant role in reciprocally regulating each other (Jüttler, Tarabin, and Schwaninger 2002). This relationship is responsible for control of body temperature, the endocrine pathways and the immune organs that are innervated by the autonomic nervous system. Research over the last few years has indicated a role of IL6 in each of these three independent pathways. IL6, along with some other inflammatory cytokines such as IL-1 β and TNF α , is one of the key mediators that is known to be involved in several different forms of pain-related disorders and injury as discussed above. The role of IL6 is of specific importance due to its ability to have both negative and positive effects on the nervous and immune systems.

One of the key challenges that needs to be addressed is the paradox surrounding the effectiveness of the therapy. Anti-IL6 therapy has been found to be successful in several human disease states but ineffective in others. One possible explanation that has been offered is the difference between the signalling of IL6 either through its classical receptor or trans-signalling via its soluble receptor (Hunter and Jones 2015; Lacroix et al. 2015).

Next generation anti-IL6 drugs in either pre-clinical and some in early clinical development are looking to selectively target IL6 trans-signalling by inhibiting sIL6R. This is of significant importance since classical IL6 signalling is required for immune homeostasis, which becomes compromised following treatments using a pan-IL6 antibody. The use of sgp130 as a selective inhibitor of IL6 trans-signalling has been shown to be successful in improving disease outcome in neuro-inflammation, allergy, arthritis, pain and cancer (Liu et al. 2016; Lissilaa et al. 2010). Anti-IL6 therapies will have to show increased consistency with their outcomes in order to be used on a larger scale in humans. For this, knowledge on why anti-IL6 therapies fail, detailed mechanisms of IL6 and its signalling pathways and specific disease conditions where anti-IL6 therapy would be more effective, need to be enhanced in order to be able to get greater efficiency.

Other neurotrophic factors as peripheral pain mediators or analgesic agents

Preclinical studies have also suggested that neurotrophic factors other than NGF may modulate nociceptive properties. However, the literature is somewhat confusing because in some cases the same factor is reported to be both pro-algesic and analgesic. The evidence for the neurotrophin BDNF is limited but reasonably clear. It is said to induce thermal hypersensitivity in rats when injected into the hind paw and following nerve injury the neutralisation of BDNF in the periphery can reduce the increase in thermal hypersensitivity (Theodosiou et al. 1999). The high affinity receptor for BDNF is TrkB and this is expressed ubiquitously in the afferent neurons innervating viscera (both nodose ganglia neurons and the relevant DRG neurons) suggesting a potential role in visceral pain states. In the adult, only about half the cutaneous nociceptors express TrkA with the remaining expressing instead c-Ret, part of the receptor complex for GDNF (Snider and McMahon 1998). When injected into the paw of naïve animals, GDNF is reported to lower thermal pain-related thresholds (Malin et al 2006). However this factor might not act as a pro-algesic mediator in persistent pain states since its application to nerve injured rats is analgesic (Boucher et al. 2000; Hoke 2014; Dong et al. 2013). Artemin, a member of the GDNF family of ligands is also able to elicit thermal hypersensitivity when given intradermally and in the CFA-induced inflammatory pain model, artemin is up-regulated (Malin, Davis, and Molliver 2007). It has also been observed that genetically modified mice which over express artemin in the skin, there is an increased sensitivity to both thermal and cold stimuli (Elitt et al. 2006). In contrast, there is a body of evidence that artemin can be analgesic in some conditions (R. Wang et al. 2014). This apparent paradox (of pro and anti-nociceptive actions of factors such as artemin and GDNF) may be explained by considering the models employed. Both these factors reverse abnormal sensory processing in neuropathic conditions – when nerves are damaged and become disconnected from their peripheral targets. Under these conditions, the sensory nerves lose their target derived trophic support and exogenous neurotrophic factors may be particularly beneficial here.

In summary, with an estimated 20% of the UK population suffering from chronic pain, there is a continuing need to discover novel therapeutic targets and inhibitors to both increase the quality of life of patients in addition to reducing the financial burden associated with health care. There is a body of pre-clinical evidence that supports a role for inhibitors of neurotrophic factors in the treatment of chronic pain, including BDNF, GDNF and artemin as discussed above. This review however, has focused mainly on NGF and IL6 as targets for treatment of chronic pain. Anti-NGF monoclonal antibody treatments such as tanuzemab are currently showing significant success as an analgesic in a number of phase II and III clinical trials for a number of chronic pain conditions such as OA. However, side effects resulting in the temporary hold on clinical trials highlight the need to further research both beneficial and potential harmful effects of anti-NGF therapies. Similarly, from the current pre-clinical data, and some limited clinical data, there is evidence that the use of IL6 inhibitors could also prove to be highly effective in the treatment of chronic pain in the future. However, with this particular inhibitor there remains a large amount of research to be done before IL6 inhibitors can be released large scale into human clinical trials.

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Figure legends

Figure 1: Schematic outlining the major anatomical pathways involved in pain processing.

Noxious stimulus is detected in the periphery and transduced into an action potential in a nociceptive neuron. Signal is then transmitted to the dorsal horn of the spinal cord where it may undergo descending or local modulation. Finally, it is transmitted up through ascending pathways of the spinal cord to the brain where it is perceived as pain.

Figure 2: NGF exerts its biological effects via TrkA and p75^{NTR}.

NGF signalling via TrkA activates the PI3K-Akt, ERK and PLC γ signalling pathways. TrkA is capable of interacting with p75^{NTR} and there is some evidence of transactivation of TrkA from G-protein coupled receptors. Activation of p75^{NTR} signals apoptosis through the JNK pathway, however, in the presence of TrkA p75^{NTR} can activate survival pathways. P75^{NTR} is capable of interactions with the sortilin, which can act as a co-receptor for proNGF signalling through p75^{NTR}. In addition there is some evidence that p75^{NTR} is capable of interacting with the Nogo receptor.

Figure 3: Mechanisms of persistent pain – peripheral sensitisation, central sensitisation and hyper-innervation

A – Schematic of peripheral sensitisation. Injury or inflammation results in the secretion of an “inflammatory soup” which includes factors such as NGF, bradykinin and PGE₂ which bind to receptors on peripheral terminals of nociceptors. This results in various processes including receptor modulation, channel modulation and gene expression which all contribute to the process of peripheral sensitisation. B- Schematic of central sensitisation. Noxious stimuli can also be amplified by processes within the CNS. The pharmacology is best understood at a spinal level, where the NMDA receptor plays a pivotal role. This form of modulation is often referred to as central sensitisation. C – Evidence of nociceptor sprouting in some models of persistent pain. This data shows sections of bone immunostained for CGRP+ sensory nerve fibres in (i) normal mice and (ii) mice with tumours implanted into intramedullary space, and (iii) mice with tumours but treated with anti-NGF. Note the NGF-dependent increase in innervation in this model. However, following anti-NGF treatment in tumour implanted mice hyper-innervation was attenuated (Jimenez-Andrade *et al*, 2011).

Figure 4: Dose dependent attenuation of pain in OA patients given anti-NGF treatment tanezumab

Patients with confirmed OA in the knee in this phase 3 clinical trial show a reduction in the pain ratings while walking compared to baseline (Lane *et al*, 2010).

Figure 5: Schematic of IL6 action in peripheral sensitisation

Similar to NGF, IL6 is secreted in response to injury or inflammation. Binding of IL6 to its receptors, gp130 and IL6R, on the surface of immune cells, for example myeloid cells, can stimulate the secretion of sensitising agents to indirectly sensitise peripheral afferents. Additionally, IL6 and sIL6R complex may also be able to directly sensitise the neurons via its interaction with neuronal gp130.

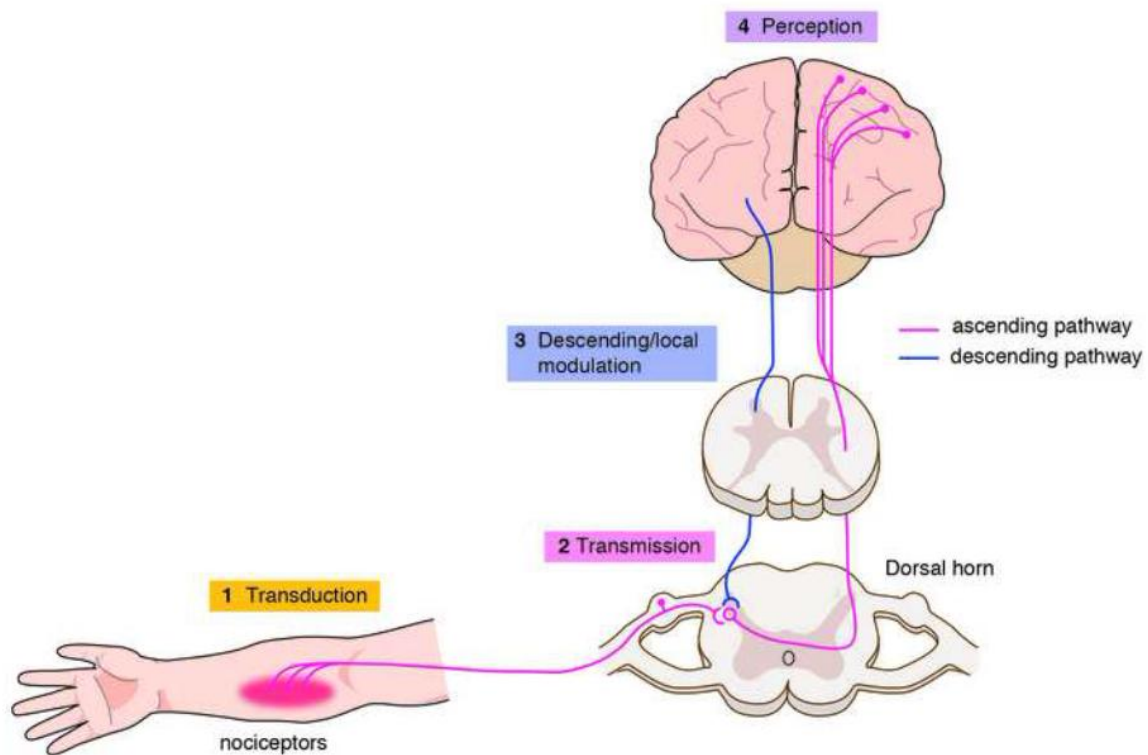


Figure 1

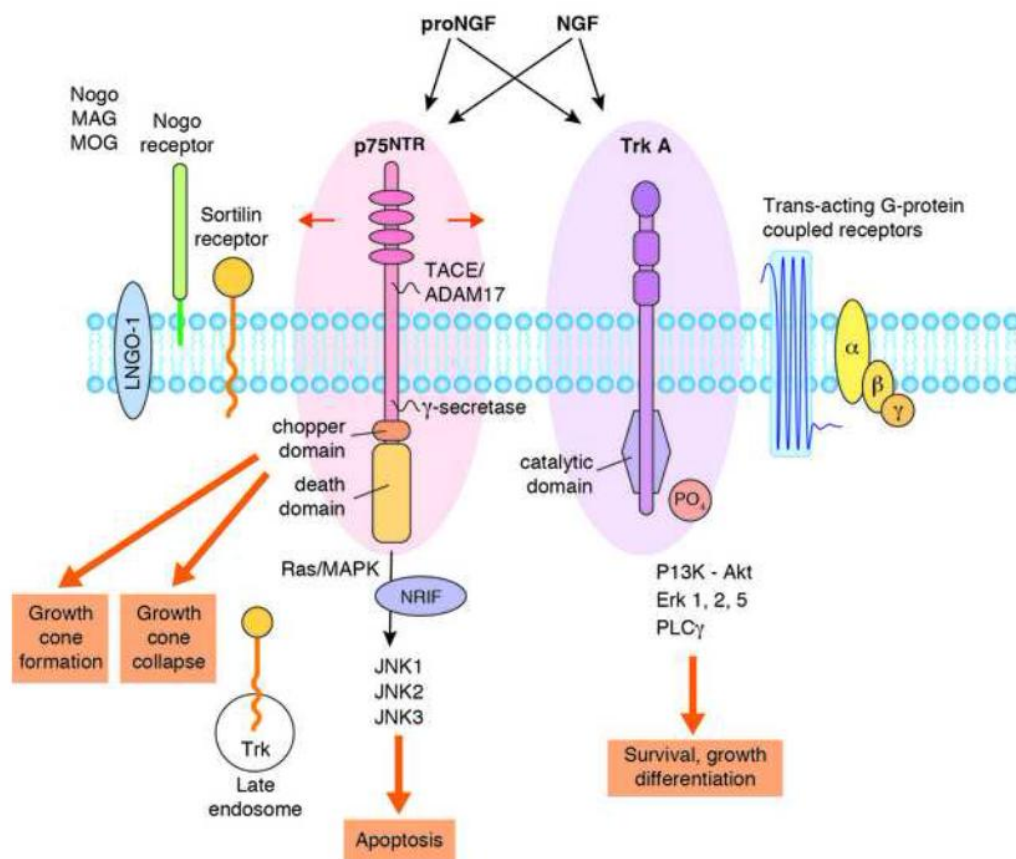


Figure 2

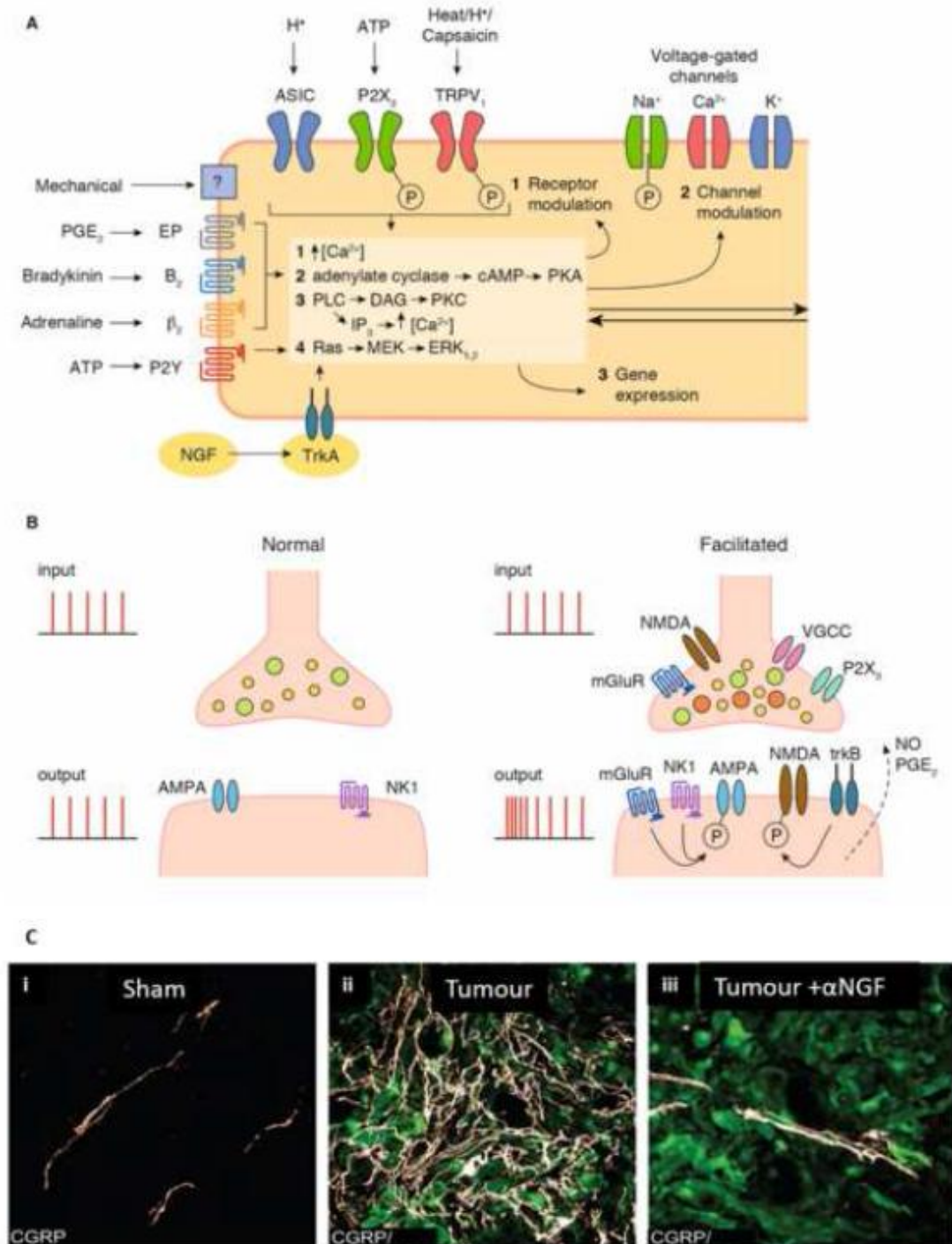


Figure 3

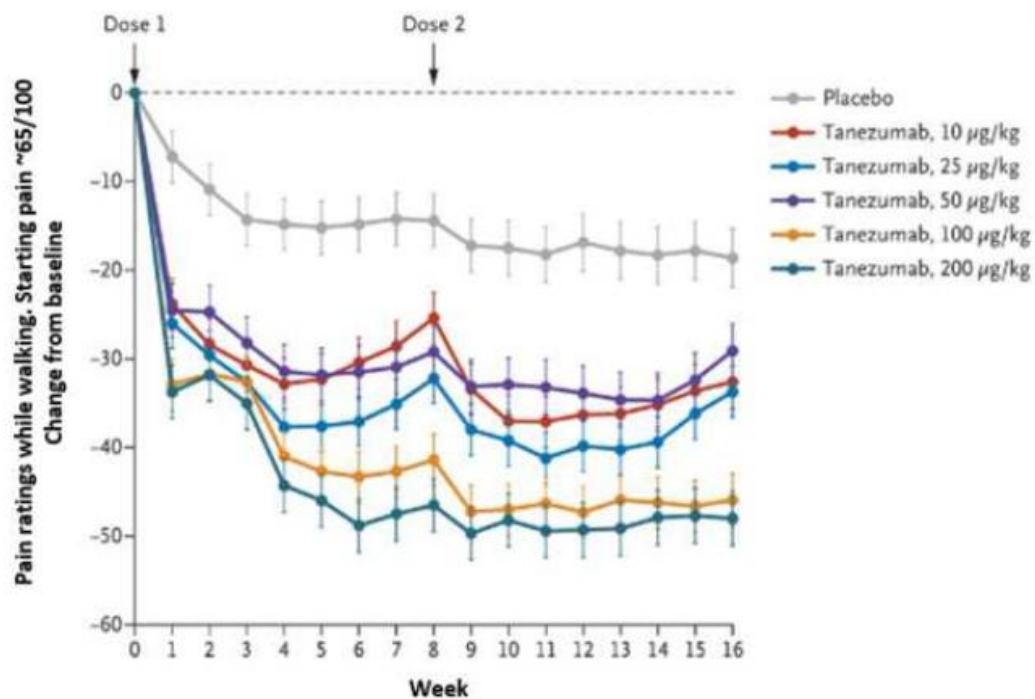


Figure 4

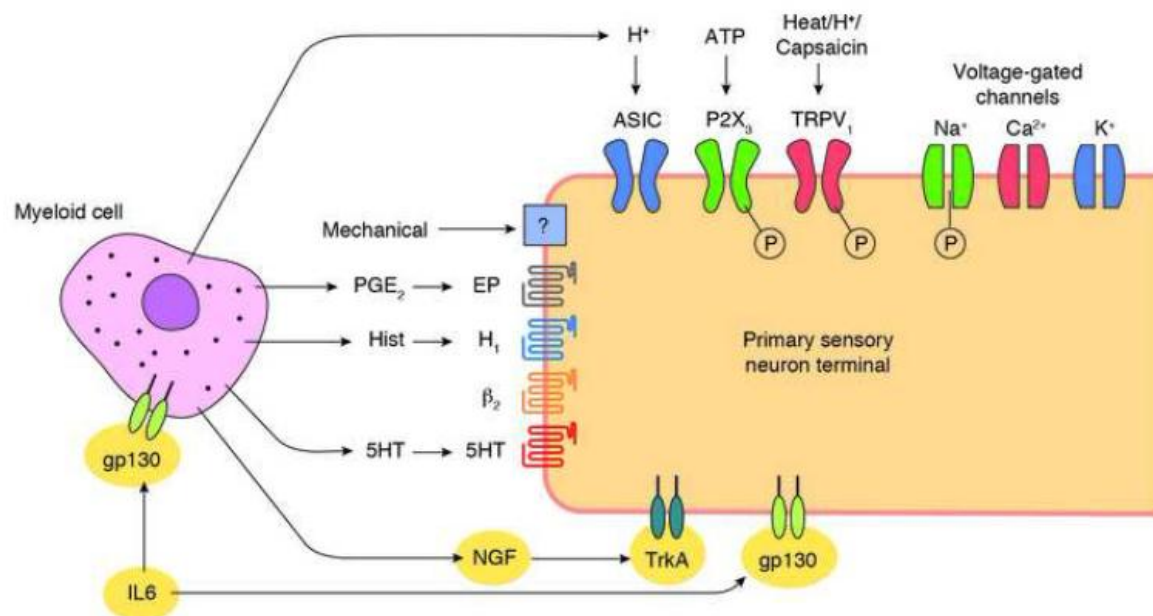


Figure 5